

b.) Amendments to the Specification<sup>1/</sup>

Please insert the following new paragraph on page 1 after line 2, before line 3.

This application is a continuation of application No. 09/496,041 filed February 2, 2000.

Please amend the paragraph starting at page 6, line 30 to page 7, line 5 to read as follows.

- (1) A process for producing a purine nucleotide which comprises: culturing in a medium a microorganism having the ability to produce a precursor of the purine nucleotide and carrying an introduced DNA which can ~~induce and~~ express an enzyme capable of synthesizing the purine nucleotide from said precursor upon induction; allowing said precursor of the purine nucleotide to accumulate in the culture; inducing ~~and expressing the expression of~~ the enzyme capable of synthesizing the purine nucleotide from said precursor and expressing said enzyme; allowing the purine nucleotide formed from said precursor to accumulate in said culture; and then recovering said purine nucleotide therefrom.

Please amend the paragraph at page 7, lines 27-33 to read as follows.

- (7) The process according to the above (1), which is characterized in that the expression of the enzyme capable of synthesizing the purine nucleotide is induced

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<sup>1/</sup> Where appropriate, e.g., where the original text is underlined, the material deleted and added herein is shown in bold.

~~and expressed~~ by the change of condition selected from the group consisting of rise in temperature, rise in pH and rise in osmotic pressure, or by the change of the carbon source from sugars to non-sugars.

Please amend the paragraphs starting at page 7, line 36 and ending at page 8, line 7 to read as follows.

- (9) A DNA which can ~~induce and~~ express an enzyme capable of synthesizing a purine nucleotide from its precursor upon induction.
- (10) The DNA according to the above (9), which can ~~induce and~~ express the enzyme capable of synthesizing the purine nucleotide upon induction by the change of condition selected from the group consisting of rise in temperature, rise in pH and rise in osmotic pressure, or by the change of the carbon source from sugars to non-sugars.

Please amend the paragraph at page 8, lines 12- 15 to read as follows.

- (13) A microorganism having the ability to produce a precursor of a purine nucleotide and carrying an introduced DNA which can ~~induce and~~ express an enzyme capable of synthesizing the purine nucleotide from said precursor said precursor.

Please amend the paragraph at page 13, lines 5-12 to read as follows.

The thus obtained gene encoding the enzyme capable of synthesizing a purine nucleotide from its precursor is inserted into an appropriate inducible-expression vector so that the gene is under the control of the transcription and translation signal capable of regulating its ~~induction and~~ expression, whereby a recombinant plasmid which can regulate the expression of said gene can be obtained.

Please amend the paragraphs at page 15, lines 6-31 to read as follows. The changes are in bold.

On the other hand, when the transcription-translation signal related to the regulation of gene expression in the inducible-expression vectors does not function in a microorganism to be used, or when it functions but the above three requirements are not sufficiently fulfilled, it is necessary to improve said transcription-translation signal region by mutation techniques or recombinant DNA techniques so that the above three requirements can be fulfilled, or to develop a new system of **induction and inducible** expression applicable to said microorganism. In the former case, the improvement can be carried out, for example, by site-directed mutagenesis referring to a transcription-translation signal sequence known for the microorganism to be used. In the latter case, the new system can be developed and constructed according to the method of developing the above general inducible-expression systems constructed for Escherichia coli, etc.

Preferred examples of the microorganisms carrying the inducible-expression system include Corynebacterium ammoniagenes strains, etc., which were respectively developed using, as the **gene-inducing and expressing inducible-gene-expression** system, a temperature-inducible plasmid vector which is prepared by ligating the P<sub>L</sub> promoter/cI857 gene carried by pPAC31 which is a vector induced at a high temperature in Corynebacterium, and pCEX2 capable of regulating the expression by addition of inexpensive acetic acid.

Please amend the paragraphs at page 16, lines 4-32 to read as follows. The changes are in bold.

The inducible-express plasmid containing the gene encoding the enzyme capable of synthesizing a purine nucleotide from its precursor can be introduced into a microorganism which produces the precursor of the purine nucleotide by using the protoplast method, ~~the electric pulse method~~ **electroporation**, the calcium chloride method, and conventional methods described in Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press (1989) (hereinafter abbreviated as Molecular Cloning, Second Edition), etc.

For example, when a bacterium belonging to the genus Corynebacterium is used as the host microorganism, the protoplast method (Japanese Published Unexamined Patent Application No. 183799/82) and ~~the electric pulse method~~ **electroporation** (Japanese Published Unexamined Patent Application No. 207791/90) are particularly effective. When Escherichia coli is used as the host microorganism, the calcium chloride method [J. Mol. Biol., 53, 159 (1970)], etc. can also be employed.

The **expression of the** enzyme capable of synthesizing a purine nucleotide from its precursor can be induced ~~and expressed~~ by culturing the thus obtained transformant of the present invention carrying the introduced gene encoding the enzyme capable of synthesizing the purine nucleotide from its precursor in an ordinary medium containing carbon sources, nitrogen sources and inorganic substances, and additionally, trace organic nutrients, as may be required, to form and accumulate the precursor of the purine nucleotide, and then subjecting the resulting culture to the induction treatment such as heating or addition of acetic acid.

Please amend the paragraph at page 20, lines 4-9 to read as follows. The changes are in bold.

The plasmid was cleaved with various restriction enzymes and analyzed, whereby it was confirmed that plasmid pLS857 comprising the XMP aminase gene **which was under the control of the P<sub>L</sub> promoter**, the cI1857 gene, the ampicillin resistance gene and the replication origin of the plasmid autonomously replicable in Escherichia coli **in the region under the control of the P<sub>L</sub> promoter** was obtained.

Please amend the paragraph at page 20, lines 28-35 to read as follows. The changes are in bold.

Corynebacterium ammoniagenes ATCC 6872 was transformed using 1 µg of the DNA obtained by the ligation treatment by means of **the electric pulse method electroporation** [Appl. Microbiol. Biotechnol., 30, 283 (1989)] and then spread on A agar medium [0.5% glucose, 1% peptone, 0.5% meat extract, 0.5% yeast extract, 0.25% sodium chloride, 1.5% agar, 10 mg/1 adenine and 10 mg/1 guanine (pH 7.2)] containing 100 µg/ml spectinomycin.

Please amend the paragraph at page 21, lines 19-23 to read as follows. The changes are in bold.

An XMP-producing strain of Corynebacterium ammoniagenes, FERM BP-1261 (Japanese Patent No. 2618383:adenine-leaky requiring and guanine-requiring), was transformed using said plasmid by the above **electric pulse method electroporation**.

Please amend the paragraph at page 24, lines 8-12 to read as follows.

To induce ~~and express~~ the expression of the XMP aminase gene, the culture broth was heated to 40°C, and kept at the same temperature with stirring (600 rpm) and aeration (1 l/min) for 6 hours, during which the pH was kept at 7.2 with 5.5 mol/l aqueous ammonia.

Please amend the paragraph at page 26, lines 23-26 to read as follows. The changes are in bold.

Corynebacterium ammoniagenes ATCC 6872 was transformed using said ligated DNA (1 µg) by **the electric pulse method electroporation**, and then spread on A agar medium containing 100 µg/ml spectinomycin.

Please amend the paragraph at page 27, lines 4-7 to read as follows. The changes are in bold.

An XMP-producing strain of Corynebacterium ammoniagenes, FERM BP-1261 (adenine-leaky requiring and guanine-requiring), was transformed using said plasmid by **the electric pulse method electroporation**.

Please amend the paragraph at page 28, lines 13-18 to read as follows.

After the fermentation was completed, in order to induce ~~and express~~ the express of the XMP aminase gene, ammonium acetate was added to the fermentation broth to a final concentration of 2%, followed by culturing with stirring (600 rpm) and aeration (1 l/min) for 10 hours, during which the pH was kept at 7.2 with 5.5 mol/l aqueous ammonia.

Please amend the paragraph at page 31, lines 6-9 to read as follows. The changes are in bold.

Corynebacterium ammoniagenes ATCC 6872 was transformed using this plasmic (1  $\mu$ g) by **the electric pulse method electroporation** method and then spread on A agar medium containing 100  $\mu$ g /ml spectinomycin.

Please amend the paragraph at page 31, lines 27-30 to read as follows. The changes are in bold.

An inosine-producing strain of Corynebacterium ammoniagenes, FERM BP-2217 (Japanese Patent No. 2578496:adenine-leaky requiring and guanine-requiring), was transformed using said plasmic by **the electric pulse method electroporation**.

Please amend the paragraph at page 34, lines 16-21 to read as follows.

After the culturing was completed, in order to induce ~~and express~~ the expression of the inosine-guanosine kinase gene, ammonium acetate was added to the obtained culture to a final concentration of 2%, following by culturing with stirring (600 rpm) and aeration (1 l/min) for 10 hours, during which the pH was kept at 7.2 with 5.5 mol/l aqueous ammonia.